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ORIGINAL RESEARCH

Integrin and gene network analysis reveals that ITGA5 and ITGB1 are prognostic in non-small-cell lung cancer

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Background: Integrin expression has been identified as a prognostic factor in non-small-cell lung cancer (NSCLC). This study was aimed at determining the predictive ability of integrins and associated genes identified within the molecular network.

Patients and methods: A total of 959 patients with NSCLC from The Cancer Genome Atlas cohorts were enrolled in this study. The expression profile of integrins and related genes were obtained from The Cancer Genome Atlas RNAseq database. Clinicopathological characteristics, including age, sex, smoking history, stage, histological subtype, neoadjuvant therapy, radiation therapy, and overall survival (OS), were collected. Cox proportional hazards regression models as well as Kaplan–Meier curves were used to assess the relative factors.

Results: In the univariate Cox regression model, *ITGA1, ITGA5, ITGA6, ITGB1, ITGB4*, and *ITGA11* were predictive of NSCLC prognosis. After adjusting for clinical factors, ITGA5 (odds ratio =1.17, 95% confidence interval: 1.05–1.31) and *ITGB1* (odds ratio =1.31, 95% confidence interval: 1.10–1.55) remained statistically significant. In the gene cluster network analysis, *PLAUR, ILK, SPP1, PXN*, and *CD9*, all associated with *ITGA5* and *ITGB1*, were identified as independent predictive factors of OS in NSCLC.

Conclusion: A set of genes was identified as independent prognostic factors of OS in NSCLC through gene cluster analysis. This method may act as a tool to reveal more prognostic-associated genes in NSCLC.

Keywords: integrin, prognosis, non-small-cell lung cancer, *ITGA5, ITGB1*

Introduction

Lung cancer, particularly non-small-cell lung cancer (NSCLC), is one of the most common malignancies and the most common cause of cancer-related mortality worldwide.[1] The prognosis of patients with NSCLC, especially in an advanced stage, is generally poor where the 5-year survival rate is <10%.[2]

Integrins are heterodimeric cell-surface adhesion receptors generally consisting of noncovalently linked alpha and beta subunits. A total of 18 alpha and eight beta subunits with different functions are currently known.[3] Integrin family members participate in a variety of processes influencing the cell's biological behavior, including cell adhesion, recognition, immune response, metastasis of tumor cells as well as embryogenesis, hemostasis, and tissue repair.[4] Alterations in integrin expression levels can influence cancer cell adhesion, polarity, and extracellular matrix assembly, which may result in tumor metastasis.[5] Integrins can also interact with tyrosine kinase receptors, such as epidermal growth factor receptor (EGFR) and vascular EGFR (VEGFR), to promote cancer cell proliferation, survival, and differentiation.[6] EGFR mutations frequently

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occur in patients with lung cancer, and these patients have been found to benefit from tyrosine kinase inhibitor-targeted therapy rather than first-line chemotherapy.[7]

There was a report that discussed the integrin profile and prognosis in NSCLC;[8] however, external validation and interactions of integrins within the network of integrins were not determined. The Cancer Genome Atlas (TCGA) database has been developed in recent years using a large amount of NSCLC RNAseq data as well as detailed clinical data, and this has made bioinformatic data mining convenient and reliable.[9] This study was aimed at determining the prognostic ability of integrins and associated genes identified through the molecular network using TCGA database analysis in NSCLC.

Materials and methods

Patients

Expression data of integrins and their associated genes and relative clinical data of patients with NSCLC were available in TCGA database provided on the website of the Cancer Genomics Browser of the University of California Santa Cruz (https://genome-cancer.ucsc.edu/). Thirty members of the integrin family were included in our study (Table S1). There were 1,092 patients with NSCLC enrolled in TCGA database (updated on February 24, 2015) according to the parameters defined in a previous study.[10] Patients without fully characterized tumors, deficient overall survival (OS) data, or incomplete RNAseq information were excluded from the study. Clinicopathological characteristics, including age, sex, histology, TNM stage, American Joint Committee on Cancer stage, smoking status, and history of neoadjuvant and radiation therapy, were collected. Information on integrin family genes was also obtained from TCGA RNAseq database. Networks of integrin genes, which were independent prognostic predictors of NSCLC, were obtained from the cBioPortal website (http://www.cbioportal.org/public-portal/cgds_r.jsp). Network filters were set as "in the same complex" or "interacted with each other", and threshold was set as >12% changes.

This study is based on publicly available data from TCGA database and did not involve interaction with human subjects or the use of personal identifying information. The study was approved by the Institutional Review Board of Guangqian Hospital, Quanzhou, Fujian, People's Republic of China.

Statistical analysis

OS was defined as time from the date of diagnosis to the date of death or the last follow-up. Patients without an event of death were recorded as censored at the time of last follow-up. The R project (3.1.3) was used to perform statistical analysis. Survival curves were constructed using the Kaplan–Meier method, with log-rank tests used to assess differences between groups. Univariate and multivariate Cox proportional hazards models were used to analyze the relationship between integrin network expression and OS of patients with NSCLC in TCGA cohort. A two-sided P-value <0.05 was considered statistically significant. Odds ratios with 95% confidence intervals (CIs) were calculated.

Results

Clinical factors in TCGA cohorts

A total of 959 patients with NSCLC, including 576 men and 383 women, from TCGA cohort were enrolled in the current study. The median age of the cohort was 67. There were 485 patients diagnosed with adenocarcinoma and 474 patients diagnosed with squamous cell carcinoma (SCC). Detailed clinicopathological data are shown in Table 1. The median OS in this cohort was 16.7 months.

ITGA5 and ITGB1 expressions were independent prognostic factors for OS in TCGA cohort

In univariate Cox regression analysis, *ITGA1, ITGA5, ITGA6, ITGB1, ITGB4*, and *ITGA11* were significantly associated with OS in patients with NSCLC (all $P<0.05$, Table 2). In multivariate models, after adjusting for age, sex, stage, histological subtype, smoking history, neoadjuvant therapy history, and radiation therapy history, *ITGA5* (HR =1.17, 95% CI: 1.05–1.31) and *ITGB1* (HR =1.31, 95% CI: 1.10–1.55) were independent predictors of prognosis (all $P<0.01$, Table 2).

We then divided TCGA cohort according to the histological subtype. In TCGA NSCLC cohort, large-cell carcinoma data were not available; therefore, we analyzed only two subgroups of adenocarcinoma and SCC. In 485 patients with adenocarcinoma, *ITGA5* (HR =1.316, 95% CI: 1.135–1.525) and *ITGB1* (HR =1.788, 95% CI: 1.399–2.286) were associated with OS in univariate analysis. However, in multivariate analysis, *ITGA6* (HR =1.208, 95% CI: 1.014–1.439) was found to be the unique, independent prognostic factor. Also, *ITGA5* (HR =1.142, 95% CI: 1.005–1.299) and *ITGB1* (HR =1.231, 95% CI: 1.006–1.507) were prognostic factors of 474 patients with SCC with univariate analysis. After adjusting for clinical factors and other integrin family members, *ITGA3* (HR =1.182, 95% CI: 1.002–1.394) was the only prognostic factor (Table 3).

Further studies of integrin and lymph node metastasis and distant metastasis were conducted with Spearman's correlation analysis. *ITGA3, ITGB5, ITGB6*, and *ITGB8* were associated with lymph node staging of SCC. *ITGB1* was the

Table 1 Clinical characteristics of patients with NSCLC in TCGA cohort

Variables	Number	%
Number of patients	959	
Age, median (range)	67	(38–90)
Sex		
Male	576	60.10
Female	383	39.90
Histology		
Adenocarcinoma	485	50.60
Squamous cell carcinoma	474	49.40
pT		
T1	270	28.20
T2	538	56.10
T3	110	11.50
T4	39	4.10
Tx	2	0.20
N		
N0	612	63.80
N1	216	22.50
N2	108	11.30
N3	7	0.70
Nx	16	1.70
M		
M0	707	73.70
M1	31	3.20
Mx	221	23.00
Stage		
I	495	51.60
II	269	28.10
III	163	17.00
IV	32	3.30
Smoking status		
Nonsmoker	86	9.00
Reformed smoker	608	63.40
Current smoker	244	25.40
History of neoadjuvant therapy		
Yes	10	1.00
No	949	99.00
History of radiation therapy		
Yes	94	9.80
No	641	66.80
Undefined	224	23.40
Median OS in months (range)	16.7	(0.5–83.3)

Note: Figures are expressed as percentage unless range (shown in parentheses).
Abbreviations: M, M stage; N, N stage; NSCLC, non-small-cell lung cancer; OS, overall survival; pT, pathological T stage; TCGA, The Cancer Genome Atlas.

Table 2 Univariate and multivariate Cox proportional hazards analysis of integrin expression and overall survival for patients with NSCLC in TCGA cohort

Gene	Univariate			Multivariate[a]		
	HR	(95% CI)	*P*-value	HR	(95% CI)	*P*-value
ITGA8	1.01	(0.96–1.06)	0.736			
ITGA9	0.99	(0.93–1.06)	0.830			
ITGA1	1.11	(1.01–1.23)	0.039*	1.10	(0.97–1.25)	0.132
ITGA2	1.07	(0.99–1.15)	0.070	1.01	(0.93–1.11)	0.760
ITGA3	1.06	(0.98–1.15)	0.148			
ITGA4	1.00	(0.91–1.10)	0.989			
ITGA5	1.21	(1.10–1.33)	0.000*	1.17	(1.05–1.31)	0.005*
ITGA6	1.09	(1.03–1.16)	0.005*	1.09	(0.98–1.22)	0.123
ITGA7	0.99	(0.91–1.09)	0.911			
ITGAX	1.00	(0.91–1.08)	0.926			
ITGAV	1.10	(0.97–1.24)	0.127			
ITGAL	0.96	(0.89–1.04)	0.338			
ITGAM	1.04	(0.97–1.12)	0.298			
ITGA2B	0.95	(0.88–1.01)	0.109			
ITGB1BP1	0.98	(0.80–1.21)	0.868			
ITGB1BP3	0.94	(0.80–1.10)	0.427			
ITGB1BP2	0.98	(0.88–1.08)	0.637			
ITGAD	0.94	(0.87–1.01)	0.091	0.94	(0.86–1.02)	0.127
ITGAE	0.97	(0.83–1.13)	0.683			
ITGBL1	1.05	(0.98–1.12)	0.149			
ITGB3BP	0.95	(0.82–1.11)	0.550			
ITGB1	1.41	(1.21–1.64)	0.000*	1.31	(1.10–1.55)	0.002*
ITGB3	1.03	(0.97–1.10)	0.271			
ITGB5	1.11	(0.98–1.27)	0.102			
ITGB4	1.11	(1.03–1.19)	0.006*	1.06	(0.97–1.17)	0.218
ITGB7	1.00	(0.90–1.10)	0.966			
ITGB6	1.06	(0.99–1.13)	0.089	1.06	(0.98–1.15)	0.127
ITGB8	1.00	(0.95–1.05)	0.932			
ITGA10	1.00	(0.93–1.09)	0.914			
ITGA11	1.07	(1.01–1.14)	0.032*	1.07	(1.00–1.16)	0.051
ITGB2	1.01	(0.93–1.09)	0.833			

Notes: [a]Multivariate Cox regression was adjusted for clinical factors (age, sex stage, histological subtype, smoking history, neoadjuvant therapy history, and radiation therapy history). *Indicates statistical significance.
Abbreviations: CI, confidence interval; NSCLC, non-small-cell lung cancer; TCGA, The Cancer Genome Atlas; HR, hazard ratio.

only factor correlated with distant metastasis in SCC. The pattern was different in adenocarcinoma. *ITGA5, ITGA7, ITGA9, ITGAD, ITGAL*, and *ITGAV* were associated with N stage, and *ITGA3, ITGB1BP3, ITGB5*, and *ITGBL1* were correlated with M stage (Table S2).

Expression levels of *ITGA5* and *ITGB1* in TCGA cohort showed nearly normal distribution (data not shown); therefore, we divided the cohort into low and high expressers according to the median expression levels of *ITGA5* and *ITGB1*. Kaplan–Meier plots demonstrated that high expressers of *ITGA5* or *ITGB1* were associated with poor OS (all $P<0.05$, Figure 1A and B). Moreover, in subgroup analysis, ITGA5 and ITGB1 were associated with poor prognosis of adenocarcinoma as well as SCC (all $P<0.05$, Figure 2A–D).

ITGA5 and ITGB1 gene cluster analysis and its association with prognosis

Although difference in integrin expression pattern existed between SCC and adenocarcinoma of lung cancer, *ITGA5* and *ITGB1* were more important genes because a selective inhibitor cilengitide has been developed.[11] Therefore, the gene networks of *ITGA5* and *ITGB1* were studied. Three situations were selected for building the interaction network of *ITGA5* and *ITGB1* (Figure 3). They were stated as "react with", "state change" (cut-point was set at 12%[12]), and "in same component". A total of 33 genes were listed

Table 3 Univariate and multivariate Cox proportional hazards analysis of integrin expression and overall survival for patients with adenocarcinoma and squamous cell carcinoma of lung cancer in TCGA cohort

Gene	Adenocarcinoma (N=485)						Squamous cell carcinoma (N=474)					
	Univariate			Multivariate[a]			Univariate			Multivariate[a]		
	HR	(95% CI)	*P*-value	HR	(95% CI)	*P*-value	HR	(95% CI)	*P*-value	HR	(95% CI)	*P*-value
ITGA1	1.138	(0.967–1.340)	0.120				1.172	(1.010–1.360)	0.037*	1.032	(0.755–1.375)	0.830
ITGA2	1.123	(1.019–1.238)	0.019*	1.044	(0.901–1.210)	0.565	0.983	(0.870–1.109)	0.776			
ITGA2B	0.920	(0.836–1.012)	0.087	0.961	(0.855–1.079)	0.502	0.973	(0.879–1.078)	0.604			
ITGA3	0.964	(0.832–1.117)	0.623				1.160	(1.043–1.290)	0.006*	1.182	(1.002–1.394)	0.047*
ITGA4	0.904	(0.777–1.051)	0.189				1.079	(0.955–1.218)	0.221			
ITGA5	1.316	(1.135–1.525)	0.000*	1.241	(0.981–1.570)	0.072	1.142	(1.005–1.299)	0.042*	1.053	(0.855–1.297)	0.626
ITGA6	1.349	(1.183–1.537)	0.000*	1.208	(1.014–1.439)	0.034*	1.046	(0.926–1.183)	0.470			
ITGA7	0.899	(0.781–1.034)	0.135				1.097	(0.971–1.240)	0.136			
ITGA8	0.950	(0.880–1.026)	0.190				1.080	(1.004–1.161)	0.040*	1.052	(0.937–1.181)	0.391
ITGA9	0.891	(0.801–0.990)	0.032*	0.934	(0.820–1.063)	0.301	1.102	(1.004–1.209)	0.042*	1.015	(0.877–1.173)	0.846
ITGA10	1.023	(0.913–1.146)	0.694				1.018	(0.902–1.150)	0.768			
ITGA11	1.114	(1.012–1.227)	0.028*	1.060	(0.926–1.214)	0.395	1.046	(0.967–1.132)	0.264			
ITGAD	0.867	(0.772–0.974)	0.016*				0.999	(0.906–1.101)	0.981			
ITGAE	0.927	(0.740–1.160)	0.507				0.996	(0.803–1.236)	0.974			
ITGAL	0.846	(0.741–0.965)	0.013*	0.836	(0.638–1.096)	0.194	1.043	(0.945–1.152)	0.399			
ITGAM	0.962	(0.860–1.075)	0.491				1.130	(1.020–1.252)	0.019*	1.092	(0.942–1.266)	0.241
ITGAV	1.235	(1.027–1.486)	0.025*	0.964	(0.738–1.259)	0.788	0.998	(0.845–1.177)	0.978			
ITGAX	0.902	(0.796–1.022)	0.106				1.097	(0.974–1.235)	0.127			
ITGB1	1.788	(1.399–2.286)	0.000*	1.191	(0.822–1.724)	0.356	1.231	(1.006–1.507)	0.044*	0.964	(0.697–1.333)	0.824
ITGB1BP1	1.105	(0.807–1.512)	0.534				0.836	(0.619–1.131)	0.245			
ITGB1BP3	0.834	(0.659–1.055)	0.131				1.155	(0.920–1.450)	0.216			
ITGB1BP2	0.985	(0.841–1.152)	0.846	0.981	(0.843–1.141)	0.801	0.978	(0.855–1.119)	0.751			
ITGB2	0.947	(0.837–1.072)	0.392				1.089	(0.969–1.223)	0.152			
ITGB3	1.023	(0.932–1.121)	0.636				1.081	(0.986–1.185)	0.098	0.890	(0.747–1.060)	0.193
ITGB3BP	1.059	(0.864–1.297)	0.583				0.828	(0.658–1.042)	0.108			
ITGB4	1.203	(1.071–1.352)	0.002*	1.029	(0.905–1.170)	0.664	1.042	(0.927–1.172)	0.488			
ITGB5	1.200	(0.962–1.498)	0.106				1.063	(0.899 –1.256)	0.474			
ITGB6	1.002	(0.883–1.138)	0.971				1.104	(1.014–1.202)	0.022*	1.019	(0.917–1.133)	0.720
ITGB7	0.884	(0.766–1.021)	0.094	0.982	(0.755–1.278)	0.894	1.117	(0.977–1.278)	0.105			
ITGB8	1.032	(0.957–1.114)	0.412				0.903	(0.816–0.999)	0.047*	0.909	(0.815–1.013)	0.083
ITGBL1	1.018	(0.904–1.148)	0.765				1.090	(1.000–1.188)	0.049*	1.031	(0.897–1.185)	0.670

Notes: [a]Multivariate Cox regression was adjusted for clinical factors (age, sex, stage, smoking history, neoadjuvant therapy history, and radiation therapy history). *Indicates statistical significance.

Abbreviations: CI, confidence interval; TCGA, The Cancer Genome Atlas; HR, hazard ratio.

in the *ITGA5* or *ITGB1* gene networks (Table S3). In the univariate Cox regression model, *PLAUR, PRKACA, ILK, YWHAZ, SPP1, PXN, LAMC1, TLN1*, and *CD9* expressions were indicated as predictive of prognosis in patients with NSCLC in TCGA cohort ($P<0.05$, Table 4). Multivariate analysis, after adjusting for all potential prognostic factors, indicated that *PLAUR* (HR =1.16, 95% CI: 1.04–1.30), *ILK* (HR =1.27, 95% CI: 1.00–1.60), *SPP1* (HR =1.08, 95% CI: 1.02–1.15), *PXN* (HR =1.25, 95% CI: 1.06–1.48), and *CD9* (HR =0.83, 95% CI: 0.74–0.94) were independent predictors of OS (all $P<0.05$, Table 4).

Discussion

Dingemans et al had reported that ITGA5 and ITGB1 were prognostic factors in the early stage of NSCLC.[8] We validated their findings in a large cohort from TCGA database. Aside from *ITGA5* and *ITGB1, PLAUR, ILK, SPP1, PXN*, and *CD9* were identified as independent predictors of OS in patients with NSCLC using gene network analysis.

As cell adhesion proteins, integrins play an important role in the cellular and extracellular environment to regulate attachment, survival, and motility.[13] Integrins are communicators between the cell and the extracellular environment.[5] Integrins can activate growth receptors and downstream cellular signals,[6] leading to cancer growth, metastasis, tumor angiogenesis, and resistance to radiotherapy and chemotherapy.[14,15] Integrin expression levels have been reported to correlate with prognosis in glioblastoma, cervical squamous cell cancer, ovarian cancer, gastric cancer, and melanoma.[13,16–23] As drug targets, integrin inhibition enhances the cytotoxic efficacy of radiation and chemotherapy.[24,25]

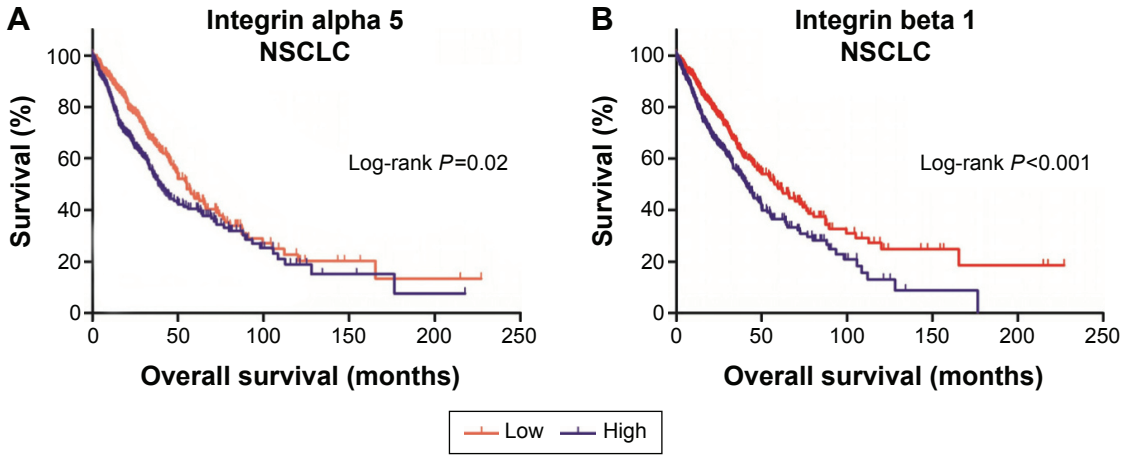

Figure 1 Kaplan–Meier plots of survival are shown according to ITGA5 and ITGB1 expression.
Notes: (**A** and **B**) Kaplan–Meier estimates of OS are shown according to the expression level of ITGA5 and ITGB1.
Abbreviations: NSCLC, non-small-cell lung cancer; OS, overall survival.

Several integrin inhibitors have entered clinical trials as cancer therapy agents.[26]

Previous studies have reported an association between increased integrin alpha 5 expression and poor outcome in NSCLC.[8,27] More specifically, Adachi et al found that in lymph-node-negative patients with NSCLC, high ITGA5 expressers had a significantly worse 5-year survival. It was suggested that tumors that express high levels of ITGA5 were more prone to metastasis or had undetectable micrometastases at the time of surgery.[27] Other studies have also pointed

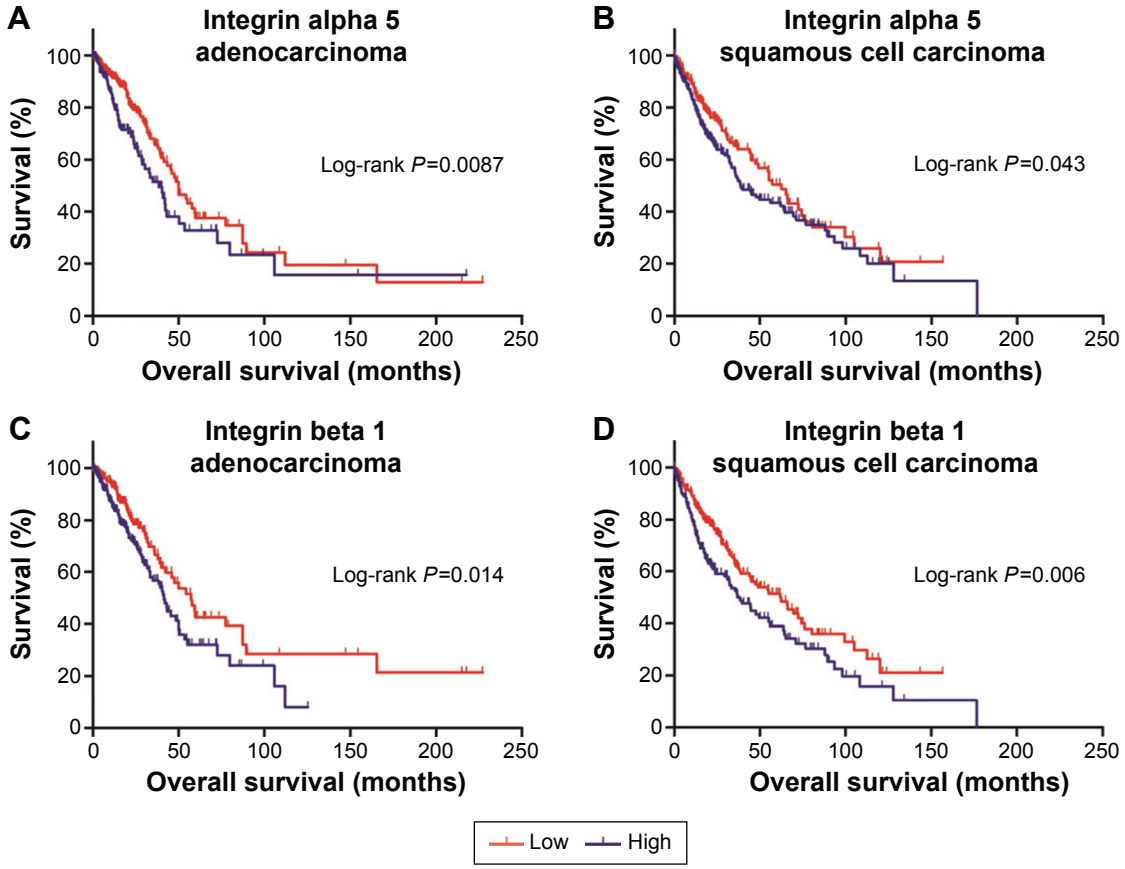

Figure 2 Kaplan–Meier estimates of overall survival according to ITGA5 expression, ITGB1 expression, and pathological histology.
Notes: (**A** and **B**) Kaplan–Meier estimates of OS were plotted according to ITGA5 expression in adenocarcinoma and squamous cell carcinoma. (**C** and **D**) Kaplan–Meier estimates of OS were demonstrated according to ITGB1 expression in adenocarcinoma and squamous cell carcinoma.
Abbreviation: OS, overall survival.

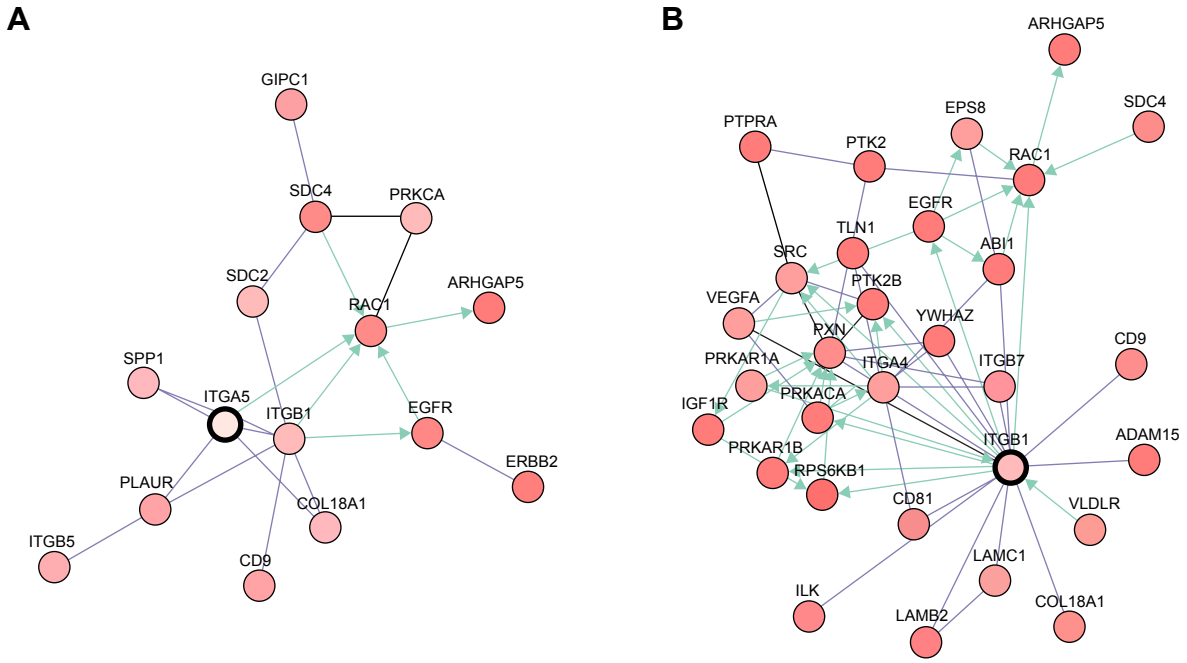

Figure 3 Interaction network building of ITGA5 and ITGB1.
Notes: The situations selected for building the networks were stated as "react with" (purple line), "in same component" (brown line), and "state change" (green arrow). The cut-off point of state change was set as 12%. (**A**) The network of ITGA5 and (**B**) the network of ITGB1.

to a relationship between ITGA5 and tumor metastasis. Valastyan et al reported that the downregulation of ITGA5 by miR-31 decreased breast cancer metastasis in vivo.[28] MiR-148b-mediated ITGA5 inhibition could also decrease lung metastasis formation.[29]

The prognostic value of ITGB1 in NSCLC has been reported,[30] and it was shown to be correlated with lymph node metastasis.[31] ITGB1 inhibition has been shown to decrease lung cancer invasion and metastasis in vitro and in vivo.[32] Our work indicates a certain correlation between NSCLC outcome and the integrin gene family; however, the mechanism, which is likely to be complex, remains unclear. Further study is required.

Our study tried to answer several questions left unanswered in previous studies. We found that integrins were differently expressed in SCC and adenocarcinoma of lung cancer. The independent factors were different as well. They were ITGA6 in adenocarcinoma and ITGA3 in SCC. The diversity of independent prognostic factors of NSCLC was possible due to different expression patterns of SCC and adenocarcinoma. A previous study had shown that ITGA3 was upregulated in adenocarcinoma but not in SCC;[33] however, we found that the upregulation of *ITGA3* in SCC indicated poor prognosis. In adenocarcinoma, the *ITGA3* levels were relatively high with minor diversity. Another controversial topic is integrin and the metastatic potential of NSCLC. In our study, different subtypes of NSCLC demonstrated diverse integrins that were associated with metastasis. Only ITGA3 and ITGA5 were associated with NSCLC metastasis. ITGA5 was associated with lymph node metastasis of adenocarcinoma, which was possibly due to the role of ITGA5 in activating endothelial cells during tumor angiogenesis.[8] Furthermore, our study identified a new set of genes as NSCLC biomarkers or even therapeutic targets through *ITGA5* and *ITGB1* network analysis. Suppression PLAUR expression could decrease lung cancer lymph node metastasis.[34] ILK binds the cytoplasmic domain of beta integrins and regulates the integrin-mediated signal transduction. Its activity is important in epithelial-to-mesenchymal transition, and the overexpression of ILK has been implicated in tumor growth and metastasis via nuclear factor-κB signaling.[35] SPP1 encodes for the protein osteopontin, which is principally expressed in NSCLC tissues. SPP1 may be tightly regulated by the Ras oncogene[36] and is important in VEGF-mediated tumor angiogenesis.[37] PXN, which encodes for the protein paxillin, has been shown to be regulated by miR-218[38] and could make EGFR-mutant lung cancers resistant to tyrosine kinase inhibitor via modulating the stability of BIM and Mcl-1 proteins.[39]

Different from the above four genes, *CD9* was a favorable factor in NSCLC outcome. This is supported by previous studies that showed that the low expression of CD9 may contribute to the early recurrence of NSCLC.[40] These studies

Table 4 Univariate and multivariate Cox proportional hazards analysis of integrin-related gene expression and overall survival for patients with NSCLC

Gene	Univariate			Multivariate[a]		
	HR	(95% CI)	*P*-value	HR	(95% CI)	*P*-value*
RAC1	1.10	(0.88–1.36)	0.395			
PLAUR	1.16	(1.05–1.28)	0.003*	1.16	(1.04–1.30)	0.010*
PRKACA	0.77	(0.60–0.98)	0.036*	0.83	(0.63–1.10)	0.196
PRKAR1A	1.06	(0.86–1.31)	0.591			
PRKAR1B	1.08	(0.94–1.24)	0.254			
PTK2	1.10	(0.89–1.37)	0.366			
ERBB2	0.96	(0.86–1.07)	0.465			
ADAM15	0.99	(0.86–1.15)	0.935			
LAMB2	1.01	(0.90–1.14)	0.855			
ABI1	1.02	(0.83–1.24)	0.886			
PTPRA	1.12	(0.89–1.40)	0.324			
ARHGAP5	0.93	(0.77–1.12)	0.437			
EPS8	0.98	(0.90–1.08)	0.707			
PRKCA	1.06	(0.96–1.16)	0.241			
ILK	1.22	(1.00–1.47)	0.046*	1.27	(1.00–1.60)	0.049*
SRC	1.11	(0.93–1.33)	0.246			
CD81	1.01	(0.84–1.21)	0.903			
YWHAZ	1.32	(1.10–1.59)	0.003*	1.20	(0.96–1.51)	0.113
IGF1R	1.07	(0.97–1.18)	0.186			
SPP1	1.09	(1.04–1.15)	0.001*	1.08	(1.02–1.15)	0.012*
PXN	1.31	(1.13–1.51)	0.000*	1.25	(1.06–1.48)	0.009*
PTK2B	0.92	(0.82–1.05)	0.212			
LAMC1	1.19	(1.03–1.37)	0.020*	1.12	(0.94–1.33)	0.197
VLDLR	0.98	(0.90–1.07)	0.662			
RPS6KB1	1.04	(0.82–1.31)	0.767			
SDC2	1.02	(0.94–1.11)	0.628			
SDC4	1.04	(0.93–1.16)	0.506			
TLN1	1.15	(0.98–1.35)	0.079*	1.19	(0.99–1.44)	0.071
VEGFA	0.99	(0.89–1.11)	0.929			
EGFR	1.05	(0.98–1.12)	0.134			
CD9	0.92	(0.84–1.00)	0.055*	0.83	(0.74–0.94)	0.003*
COL18A1	1.08	(0.98–1.20)	0.137			
GIPC1	1.01	(0.86–1.17)	0.925			

Notes: [a]Multivariate Cox regression was adjusted for clinical factors (age, sex, stage, histological subtype, smoking history, neoadjuvant therapy history, and radiation therapy history). *Indicates statistical significance.
Abbreviations: CI, confidence interval; EGFR, epidermal growth factor receptor; NSCLC, non-small-cell lung cancer; HR, hazard ratio.

suggested that the influence of integrins on the outcome of NSCLC might be via the regulation of epithelial-to-mesenchymal transition, tumor invasion, angiogenesis, and metastasis. These consistent results demonstrated that our method was applicable for detecting new prognostic indicators or even therapeutic targets.

In the study, all information was obtained from a large population with long-time follow-up and standard specimen collection and sequencing. The results were open-access, repeatable, and with high statistical power. However, there were certain limitations to our study. Although there was external validation previously,[8] we analyzed the correlation among integrins and network gene expression and NSCLC OS only in TCGA cohort. The prognosis of NSCLC is affected by many factors, such as comorbidity, tumor stage, surgical performance, and response to radiation therapy and chemotherapy, so a single biomarker is not enough. In addition, information on ethnicity was not available in TCGA database. In conclusion, further mechanistic research will be required to understand in more detail the integrin family and its role in patients with NSCLC.

Conclusion

ITGA5 and *ITGB1* were identified as independent prognostic integrin markers associated with OS in NSCLC, and several outcome-related genes were determined through gene cluster analysis. This method could act as a tool to uncover more prognostic-associated genes and therapeutic targets in NSCLC.

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Disclosure

The authors declare no conflicts of interest in this work.

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Supplementary materials

Table S1 Gene IDs of integrin family and related genes

Official gene symbol	Full name	UniGene
ITGA1	Integrin, alpha 1	Hs.644352
ITGA2	Integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)	Hs.482077
ITGA2B	Integrin, alpha 2b (platelet glycoprotein IIb of IIb/IIIa complex, antigen CD41)	Hs.411312
ITGA3	Integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor)	Hs.265829
ITGA4	Integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor)	Hs.440955
ITGA5	Integrin, alpha 5 (fibronectin receptor, alpha polypeptide)	Hs.505654
ITGA6	Integrin, alpha 6	Hs.133397
ITGA7	Integrin, alpha 7	Hs.524484
ITGA8	Integrin, alpha 8	Hs.171311
ITGA9	Integrin, alpha 9	Hs.113157
ITGA10	Integrin, alpha 10	Hs.158237
ITGA11	Integrin, alpha 11	Hs.436416
ITGAD	Integrin, alpha D	Hs.679163
ITGAE	Integrin, alpha E (antigen CD103, human mucosal lymphocyte antigen 1, alpha polypeptide)	Hs.513867
ITGAL	Integrin, alpha L (antigen CD11A (p180), lymphocyte function-associated antigen 1, alpha polypeptide)	Hs.174103
ITGAM	Integrin, alpha M (complement component 3 receptor 3 subunit)	Hs.172631
ITGAV	Integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51)	Hs.436873
ITGAX	Integrin, alpha X (complement component 3 receptor 4 subunit)	Hs.248472
ITGB1	Integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)	Hs.643813
ITGB1BP1	Integrin, beta 1 binding protein 1	Hs.467662
ITGB1BP2	Integrin, beta 1 binding protein (melusin) 2	Hs.109999
ITGB1BP3	Integrin, beta 1 binding protein 3 (nicotinamide riboside kinase 2)	Hs.135458
ITGB2	Integrin, beta 2 (complement component 3 receptor 3 and 4 subunit)	Hs.375957
ITGB3	Integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)	Hs.218040
ITGB3BP	Integrin, beta 3 binding protein (beta 3-endonexin)	Hs.166539
ITGB4	Integrin, beta 4	Hs.632226
ITGB5	Integrin, beta 5	Hs.536663
ITGB6	Integrin, beta 6	Hs.470399
ITGB7	Integrin, beta 7	Hs.654470
ITGBL1	Integrin, beta-like 1 (with EGF-like repeat domains)	Hs.696554

Table S2 Spearman's correlation analysis of integrin family and N stage and M stage of NSCLC

Gene	Squamous cell carcinoma				Adenocarcinoma			
	N (N=468)		M (N=394)		N (N=475)		M (N=344)	
	Coefficient	*P*-value*	Coefficient	*P*-value*	Coefficient	*P*-value*	Coefficient	*P*-value*
ITGA1	0.016	0.733	0.072	0.153	0.065	0.156	–0.025	0.643
ITGA2	–0.047	0.308	0.039	0.443	0.074	0.105	–0.015	0.779
ITGA2B	0.034	0.469	0.006	0.911	–0.070	0.129	–0.003	0.956
ITGA3	–0.098	**0.035**	0.041	0.412	0.051	0.264	–0.107	**0.047**
ITGA4	0.013	0.780	0.001	0.988	–0.068	0.140	–0.084	0.118
ITGA5	–0.068	0.142	0.049	0.328	0.113	**0.014**	–0.019	0.721
ITGA6	–0.033	0.471	–0.032	0.532	0.003	0.943	0.030	0.574
ITGA7	–0.016	0.738	–0.070	0.166	–0.134	**0.003**	–0.035	0.514
ITGA8	–0.052	0.261	0.064	0.205	–0.086	0.062	–0.048	0.370
ITGA9	–0.028	0.546	–0.018	0.714	–0.132	**0.004**	–0.035	0.517
ITGA10	–0.055	0.237	0.002	0.972	–0.083	0.070	0.080	0.139
ITGA11	0.017	0.709	0.048	0.343	0.075	0.105	–0.097	0.072
ITGAD	–0.032	0.489	–0.058	0.248	–0.154	**0.001**	–0.053	0.326
ITGAE	0.090	0.051	–0.084	0.097	–0.047	0.304	–0.043	0.427
ITGAL	0.012	0.790	–0.056	0.269	–0.102	**0.026**	–0.069	0.203
ITGAM	–0.027	0.559	–0.036	0.473	–0.037	0.422	–0.085	0.116
ITGAV	–0.088	0.058	0.089	0.077	0.092	**0.045**	–0.070	0.192
ITGAX	–0.037	0.420	–0.004	0.929	–0.072	0.116	–0.050	0.353
ITGB1	–0.040	0.384	0.111	**0.028**	0.046	0.319	0.000	0.993

(*Continued*)

Table S2 (*Continued*)

Gene	Squamous cell carcinoma				Adenocarcinoma			
	N (N=468)		M (N=394)		N (N=475)		M (N=344)	
	Coefficient	*P*-value*	Coefficient	*P*-value*	Coefficient	*P*-value*	Coefficient	*P*-value*
ITGB1BP1	0.080	0.083	0.068	0.179	0.054	0.242	0.006	0.917
ITGB1BP3	–0.026	0.577	–0.077	0.125	–0.077	0.095	0.128	**0.018**
ITGB1BP2	–0.089	0.054	–0.014	0.776	–0.011	0.814	–0.088	0.105
ITGB2	0.012	0.802	–0.062	0.220	–0.027	0.562	–0.099	0.067
ITGB3	–0.079	0.086	0.035	0.488	0.071	0.122	–0.071	0.187
ITGB3BP	–0.042	0.366	–0.019	0.702	–0.021	0.641	0.097	0.072
ITGB4	0.007	0.872	0.062	0.218	0.076	0.096	–0.040	0.465
ITGB5	–0.094	**0.041**	0.059	0.245	0.022	0.626	–0.114	**0.035**
ITGB6	–0.140	**0.002**	0.080	0.112	–0.003	0.953	–0.033	0.548
ITGB7	0.016	0.734	–0.083	0.099	–0.063	0.173	–0.083	0.126
ITGB8	–0.116	**0.012**	0.029	0.566	0.009	0.852	–0.036	0.502
ITGBL1	–0.005	0.914	0.082	0.106	–0.070	0.126	–0.115	**0.032**

Note: *Bold type indicates statistical significance.
Abbreviations: M, M stage; N, N stage; NSCLC, non-small-cell lung cancer.

Table S3 Gene IDs of ITGA5 and ITGB1 network genes

Official gene symbol	Full name	UniGene
ABI1	abl-interactor 1	Hs.508148
ADAM15	ADAM metallopeptidase domain 15	Hs.312098
ARHGAP5	Rho GTPase-activating protein 5	Hs.592313
CD81	CD81 molecule	Hs.54457
CD9	CD9 molecule	Hs.114286
COL18A1	Collagen, type XVIII, alpha 1	Hs.517356
EGFR	Epidermal growth factor receptor	Hs.488293
EPS8	Epidermal growth factor receptor pathway substrate 8	Hs.591160
ERBB2	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2	Hs.446352
GIPC1	GIPC PDZ domain containing family, member 1	Hs.655012
IGF1R	Insulin-like growth factor 1 receptor	Hs.643120
ILK	Integrin-linked kinase	Hs.706355
LAMB2	Laminin, beta 2 (laminin S)	Hs.439726
LAMC1	Laminin, gamma 1 (formerly LAMB2)	Hs.609663
PLAUR	Plasminogen activator, urokinase receptor	Hs.466871
PRKACA	Protein kinase, cAMP-dependent, catalytic, alpha	Hs.631630
PRKAR1A	Protein kinase, cAMP-dependent, regulatory, type I, alpha	Hs.280342
PRKAR1B	Protein kinase, cAMP-dependent, regulatory, type I, beta	Hs.520851
PRKCA	Protein kinase C, alpha	Hs.531704
PTK2	PTK2 protein tyrosine kinase 2	Hs.395482
PTK2B	PTK2B protein tyrosine kinase 2 beta	Hs.491322
PTPRA	Protein tyrosine phosphatase, receptor type, A	Hs.269577
PXN	Paxillin	Hs.446336
RAC1	Ras-related C3 botulinum toxin substrate 1	Hs.413812
RPS6KB1	Ribosomal protein S6 kinase, 70 kDa, polypeptide 1	Hs.463642
SDC2	Syndecan 2	Hs.1501
SDC4	Syndecan 4	Hs.632267
SPP1	Secreted phosphoprotein 1	Hs.313
SRC	v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian)	Hs.195659
TLN1	Talin 1	Hs.471014
VEGFA	Vascular endothelial growth factor A	Hs.73793
VLDLR	Very low density lipoprotein receptor	Hs.370422
YWHAZ	Tryptophan 5-monooxygenase activation protein, zeta polypeptide	Hs.492407

Abbreviation: EGFR, epidermal growth factor receptor.

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